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(54) Title: HIGH SUGAR DOUGH YEAST STRAINS

(57) Abstract

The present invention provides an improved strain of bakers yeast *Saccharomyces cerevisiae* which shows enhanced sugar tolerance, such as increased rates of CO_2 production in doughs containing high amounts of solubles such as sugar. This strain has been designated SDG12 and a sample of this yeast strain has been deposited with Australian Government Analytical Laboratories, 1 Suakin Street Pymble, New South Wales 2073, Australia under the Budapest Treaty and has been accorded Accession No. N95/32800. Samples of this strain are available from this International Depository in accordance with the provisions of the Budapest Treaty.

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HIGH SUGAR DOUGH YEAST STRAINS.

The present invention relates to improved strains of bakers yeast *Saccharomyces cerevisiae* which show enhanced sugar tolerance, such as increased rates of CO_2 production in doughs containing high amounts of solubles such as sugar.

Bakers yeast is typically produced by a fed batch fermentation process, and the product used in the leavening of doughs. Yeast doughs can be classified, according to relative amounts of sugars present in them, ranging from "lean" doughs, with no added sugar, "low-sugar" doughs, with a sugar content of 2 to 6% by weight, to "medium-sugar" doughs, with a sugar content of 6 to 13%, and "high-sugar" doughs with a sugar content above 13%. In the majority of baked products in Europe and America the concentration of added sugar to doughs is low or medium (on average 8.2% in the USA, and typically ranges from 0% to 13% w/w). In East Asian countries the majority of bread consumed contains high or very high concentrations of sugar, up to 40% (w/w). In high-sugar doughs, such as those used in East Asia, yeast strains show a significant reduction in fermentation rate and a corresponding delay in the time required to leaven the dough. Since the rate of bread consumption is rapidly increasing in East Asia, the availability of yeast capable of performing well in high sugar doughs is of increasing industrial importance.

In order to produce bakers yeast with high sugar tolerance, the specialist in the field has the choice between, on the one hand modifying the process of cultivation of yeast and, on the other hand, obtaining novel strains of yeast.

It is well known in the art of yeast propagation that high osmotic pressure caused by the addition of non-nutritive ionic salts, generally halides, sulfates, or carbonates of elements from Groups 1a and 2a of the Periodic Chart, may significantly enhance the activity of yeast in sweet doughs. It is also well known that high concentration of salts in a yeast-growing medium at concentrations that improve sugar tolerance can magnify one property more or less to the detriment of another, such as substantial retardation of the growth-rate of yeast cells, resulting in low yields. It is further known that such improvements achieved by modifying growth conditions of yeast can only increase the

activity of osmo-sensitive yeast to a small extent. Attempts to produce osmotolerant yeasts by modifying yeast growth conditions are disclosed in U.S. Patent. Nos. 1,727,847, 3,617,306, and 4,420,563. The disadvantage of these methods are the relatively low level of osmotolerance achieved, the strain dependence of that property, the difficulties to put into practice reproducibly, and lowered productivity and profitability of the production process.

The development of novel strains of inherently osmotolerant baking yeast strains that also maintain other industrial characteristics such as high productivity is therefore the best practical solution, especially since the employment of specific cultivation conditions can only reinforce the natural properties possessed by the yeast strain.

The present inventors have produced a bakers yeast *Saccharomyces cerevisiae* strain which is improved in respect to gas production in 16% and 25 % sugar doughs. This strain has been designated SDG12 and a sample of this yeast strain was deposited with Australian Government Analytical Laboratories, 1 Suakin Street Pymble, New South Wales 2073, Australia on 2 May 1995. This deposit was converted to a deposit under the Budapest Treaty on 29 May 1995 and was accorded Accession No. N95/32800. Samples of this strain are available from this International Depository in accordance with the provisions of the Budapest Treaty.

Accordingly, in a first aspect the present invention consists in a substantially pure culture of bakers yeast *Saccharomyces cerevisiae* strain SDG12 (AGAL N95/32800).

In a second aspect the present invention consists in bakers yeast *Saccharomyces cerevisiae* strains derived from yeast strain SDG12 (AGAL N95/32800).

In a third aspect the present inventions consists in a fresh or dry bakers yeast composition, the compositions being characterised in that it includes bakers yeast *Saccharomyces cerevisiae* strain SDG12 (AGAL N95/32800).

In a fourth aspect, the present inventions consists in a frozen baker's yeast composition, the composition being characterised in that it includes bakers yeast *Saccharomyces cerevisiae* strain SDG12 (AGAL N95/32800).

In order that the nature of bakers yeast *Saccharomyces cerevisiae* strain SDG12 may be more clearly understood its performance was compared with other yeast strains, strains A and B, representative of those currently used in markets where tolerance of high sugars is important. These two strains (A and B) are sold under the names "Mauripan Gold" and "Saf.Instant", respectively. These comparisons are followed by a list of further characteristics of strain SDG12.

Generation of yeast strain SDG12

Hybrid yeasts were produced using a "mass mating strategy". In a typical procedure, the parent strains were sporulated by incubation at 20°C on a solid medium comprising 0.5% w/v acetate and 1% agar. After 5-6 days asci were harvested and spores recovered by the ether method of Dawes and Hardie (Molec. Gen. Genet., 131: 281-289 1974). In this method, the un-sporulated vegetative cells are killed by ether. Spores were separated from any remaining vegetative cells by mixing the suspension with an equal volume of mineral oil then allowing the spores to partition into the oil phase. They were subsequently germinated by spreading to YEPD agar plates. The haploid colonies thus generated were resuspended in YEPD, mixed with haploids from other selected parent strains and the haploids allowed to randomly mate to each other for 24 hours. After mating, the mixed population of strains were spread onto YEPD agar plates and incubated for 48 hours at 30°C to allow single isolated colonies of yeast to form. Isolated single colonies of yeast were picked and streaked onto fresh YEPD plates prior to assessment in the Laboratory Scale Activity Test. In the Laboratory Scale Activity test, the activity of the hybrids were compared to the activity of strains A and B, which are commonly used commercially in high sugar dough applications. Strain SDG12 was identified in the laboratory scale activity test as baker's yeast with outstanding sugar tolerance.

Assessment of Strain SDG12

Laboratory Scale Activity Test.

Baking performance of yeast strains is predicted in the laboratory by growing strains in synthetic medium (CM medium) and testing them in high sugar doughs. In this test, the culture is inoculated into 200 ml of 0.5% CM medium contained within a 1 L Erlenmeyer flask. The culture is incubated 16 hours at 30°C in an orbital shaker at 200 r.p.m. The OD_{640} of the culture is measured and the following formula used to calculate the inoculum size for the test culture.

$$\text{Volume} = (16/\text{OD}) \times 2.5$$

The calculated inoculum is added to 4, 2 L Erlenmeyer flasks containing 500 ml of 0.5% CM medium. The cultures are incubated for exactly 24 h at 30°C in an orbital shaker at 200 r.p.m. Prior to harvesting, the concentration of ethanol in the supernatant is measured using a Gas Chromatograph (Column; S.G.E. (Aust) BP21 (polyethylene glycol), 0.25 micron film, 0.32 mm internal diameter, 25 m length. Detector; F.I.D Injection; split 30:1. Carrier gas: H_2 (8 p.s.i). Injector temperature; 180°C. Column temperature; 150°C. Detector temperature; 200°C.). The cultures are harvested when concentrations of ethanol are below 0.01% w/v. The cultures are harvested by centrifugation and the supernatant is removed. The activity of 10 g of the harvested yeast is measured in 16% and 25% sugar doughs as described in Table 1. All activity tests were carried out using a Risograph (RDesign w. 700 Main St. Pullman WA 99163) set at 30°C using the dough formulations shown in table 1.

CM composition

Compound	Conc (g/l)
Sucrose	5.0
KH_2PO_4	0.5
$(NH_4)SO_4$	0.75
NaCl	0.1
$CaCl_2 \cdot 2H_2O$	0.1
$MgSO_4 \cdot 7H_2O$	0.5
citric acid	3.415
tri sodium citrate	8.896
Compound	Conc (mg/l)
Ca pantothenate	1.0
thiamine	1.0
pyroxidine·HCl	1.0
inositol	2.0
nicotinic acid	0.5
biotin	0.2
ferric citrate·$5H_2O$	6.05
$CuSO_4 \cdot 5H2O$	0.2
$ZnSO_4 \cdot 7\ H_2O$	0.5
$MnSO_4 \cdot 4H2O$	1.0
$Na_2MoO_4 \cdot 4H20$	0.5
Na_2B_4O7	0.5

YEPD composition

Compostition	% (w/v)
Yeast extract	0.5%
Peptone	1.0%
KH2PO4	0.3%
Glucose	2%

Table 1- Formulations used in Laboratory Scale Activity Test.

Ingredients	16% Sugar dough	18% Sugar dough	25% Sugar dough
Flour	250 g	250 g	250 g
Sugar	40 g	45 g	62.5 g
Bread improver*	1.3 g	1.3 g	1.3 g
Water	91 ml	91 ml	92 ml
Salt solution (9.5% w/v)	27 ml	27 ml	27 ml
Yeast (at 30% solids)	10 g	10 g	10 g

* Mauri Foods Bakerine special.

Results

The data obtained (Table 2) show that the yeast strain SDG12 performs significantly better in both the 16% and 25% high sugar doughs than either of the commonly used industrial strains, A and B. The increase in gassing rate is in the order of 15% in the 16% sugar dough and 20% in the 25% sugar dough.

Table 2 Activity Comparison

Strain	Total ml CO_2 produced in 16% sugar dough*		Total ml CO_2 produced in 25% sugar dough*	
	60 min	120 min	60 min	120 min
A	671	1656	391	1051
B	590	1479	334	921
SDG12	775	1916	454	1284

*All activities corrected to 30% solids and 50% protein.

Freeze Thaw Stability

The resistance of strain SDG12 and control strain B to two cycles of freeze/thawing was evaluated using yeast produced under industrial conditions. The yeast was grown in a standard yeast manufacturing process, as described by G. Read., T. W. Nagodawithana. Baker's yeast production, pp 261-350. *In* Yeast Technology. 1991. Van Nostrand Reinhold New York. 500g of compressed yeast at 33% solids was frozen at -20°C. After three days, the blocks of yeast were thawed (1hr at 4°C) and the activity of the yeast evaluated. For the second freeze/thaw cycle the yeast was frozen again at - 20°C and thawed after further 10 days of storage at -20°C. The activity of 10 g of the yeast samples was measured in 18% and 25% sugar doughs as described in Table 1. All activity tests were carried out using a Risograph (RDesign w. 700 Main St. Pullman WA 99163) set at 30°C using the dough formulations shown in Table 1.

Results

The data obtained (Table 3) show that the SDG12 strain performs significantly better in both the 18% and 25% high sugar doughs after freeze/thawing than the commonly used industrial strain B. After two cycles of freeze thawing, strain SDG12 was 15% more active in 18% sugar dough, and 17% more active in 25% sugar dough. than strain B.

Table 3. Freeze Thaw Resistance

Treatment	Dough type	Strain SDG12 (ml CO_2 in 2 hours)	Strain B (ml CO_2 in 2 hours)
Fresh	18% sugar dough	1710	1460
	25 % sugar dough	1280	1050
1st freeze thaw cycle	18% sugar dough	1605	1345
	25% sugar dough	1110	980
2nd freeze thaw cycles	18% sugar dough	1515	1315
	25% sugar dough	915	915

Microbiological Strain Characterisation

Strain SDG12 was characterised according to the method in Heard and Fleet (J Appl. Bacteriol., 68: 447-451, 1990) and is identified as *Saccharomyces cerevisiae* Meyen ex Hansen (1883). This identification is also supported by the data given by Barnett, Payne and Yarrow (Yeasts: characteristics and identification. 2nd ed., 1990).

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:

1. A substantially pure culture of bakers yeast *Saccharomyces cerevisiae* strain SDG12 (AGAL N95/32800).

2. Bakers yeast *Saccharomyces cerevisiae* strains derived from yeast strain SDG12 (AGAL N95/32800).

3. A fresh or dry bakers yeast composition, the compositions being characterised in that it includes bakers yeast *Saccharomyces cerevisiae* strain SDG12 (AGAL N95/32800).

4. A frozen baker's yeast composition, the composition being characterised in that it includes bakers yeast *Saccharomyces cerevisiae* strain SDG12 (AGAL N95/32800).

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 2, line 15

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution: AUSTRALIAN GOVERNMENT ANALYTICAL LABORATORIES

Address of depositary institution *(including postal code and country)*:

1 Suakin Street, Pymble, New South Wales, 2073, Australia

Date of deposit	Accession Number
29 May 1995	N95/32800

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

Prior to grant, lapsing or refusal of the application, samples of the microorganism are only to be made available to an independent expert. (EPC Rule 28(4)) (AU Reg. 3.25(3)).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00334

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 1/18, C12R 1/865

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
WPAT and CHEM ABS
See details in electronic database box below.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT WPAT, CHEM ABS DATABASES; KEYWORDS; YEAST#, SACCHAROMYCES (W) CERIVISIAE, HIGH (5N) SUGAR (5N) DOUGH#, SWEET OR SUGAR (5N) TOLERAN:, GAS (5N) PRODUCT:, (SUCROSE OR SUGAR) (5N) (TOLERAN ? or TOLERAT?)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AU 67431/87 (UNIVERSAL FOODS CORPORATION) pubished 16 July 1987	
A	AU 29214/84 (UNIVERSAL FOODS CORPORATION) published 13 December 1984	
A	Derwent Abstract Accession No. 95-157838/21, Class D11, D16, JP 07079767-A ((KANF) KANEBUCHI KAGAKU KOGYO KK) 28 March 1995 (28.03.95)	

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

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Date of the actual completion of the international search	Date of mailing of the international search report
	5 SEP 1996

Name and mailing address of the ISA/AU	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00334

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Derwent Abstract Accession No. 94-068195/09, Class D11, D16, JP 06000052-A ((SANY) SANKO CO LTD; (SANK-) SANK YO FOODS KK) 11 January 1994 (11.01.94)	
A	Derwent Abstract Accession No. 93-338912/43, Class D11, D16, JP 05244934-A ((NIIO) NIPPON SANSO KK), 24 September 1993 (24.09.94)	

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 96/00334

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
AU	67431/87	EP	229976	DE	3684444	CA	1306960
AU	29214/84	EP	128524				

END OF ANNEX